899-Pos

Simulated Self-Assembly of Photosynthesis Proteins in Stacked Thylakoid Membranes

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Plants are able to tune their photosynthetic efficiency in response to large and rapid fluctuations in the quality and quantity of light. Adaptation mechanisms such as state transitions and the PSII repair cycle are largely structural, involving dynamic changes in the self-assembled distribution of photosystem (PSII) and light-harvesting (LHCII) pigment-protein complexes between stacked and unstacked regions of the thylakoid membrane. Although thylakoid grana stacks are among the most crowded biomembranes in nature, dramatic fluxes of LHCII over hundreds of nanometers can take place in a matter of minutes. The driving force and molecular mechanism behind these structural transitions are unclear. We present a coarse-grained model of protein-protein interactions in stacked grana thylakoid membranes, and simulations of the self-assembly of densely packed PSII and LHCII in changing environmental conditions.

900-Pos

Specific Chromophore-Protein Interactions in Bacteriophytochromes Rpbphp2 and Rpbphp3 from Rhodopseudomonas Palustris

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Various organisms can sense light through a large family of signaling proteins known as photoreceptors. Upon absorption of a photon in the appropriate wavelength range, photoreceptors undergo structural changes in the chromophore, an organic pigment embedded in the photosensory module of the protein. Phytochromes are red-light photoreceptors originally discovered in plants and more recently in bacteria. They are unique in their ability to undergo reversible photoconversion between two photoisomerizable states, Pr (red light ~ 700 nm) and Pfr (far-red light ~ 750 nm). In the photosynthetic bacterium, *Rhodopseudomonas palustris,* a pair of bacteriophytochromes, RpBphP2 (P2) and RpBphP3 (P3) modulate synthesis of a light-harvesting complex. P2 and P3 have the same biliverdin chromophore (BV) and share 52% amino acid sequence identity, yet they have distinct photoconversion properties. P2, similar to classical bacteriophytochromes, alternates between Pr and Pfr states. P3 is unusual since it alternates between Pr and a unique Pnr (near-red light ~ 650 nm) state. Our experimental goal is to identify amino acid residues that form specific interactions with BV during photoconversion and as such play key roles in forming distinct photoisomerizable states of P2 and P3. Through site-directed mutagenesis of conserved amino acids informed by structural and sequence analysis of bacteriophytochromes, we created a single-amino acid mutant variant of P3 T480P that undergoes classic reversible photoconversion between Pr and Pfr states. We also report on P2 and P3 mutants that show minimal absorption in the red region of visible light spectrum or are naturally more fluorescent than wild-type proteins. Currently, we are investigating chemical mechanisms that justify observed P2 and P3 mutant phenotypes.

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Cyclic Electron Transfer and Photosynthetic Energy Balance

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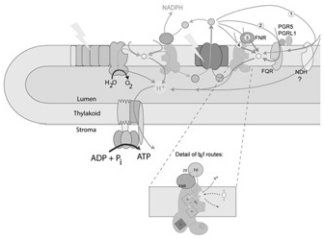
Cyclic electron flow around photosystem I (CEF1) is thought to balance chloroplast ATP/NADPH output to match downstream demands, but despite decades of work the regulation, pathway, catalytic and proton pumping capacity of the process remain controversial. At least four different pathways have been proposed for PQR, through NAD(P)H:plastoquinone reductase (NDH (1)), the antimycin A-sensitive ferredoxin:plastoquinone reductase (FQR (2)), which involved the protein PGR5, through ferredoxin:NADP+ oxidoreductase (FNR, (3)) and through the cytochrome b_6f complex (4). In addition, neither the capacity of CEF1, or the system that regulates it are known. We isolated a new class of Arabidopsis mutant, *hcef* for high CEF1, which shows dramatically higher CEF1. Characterization of these mutants shows that 1) elevated CEF1 flows through NDH rather than other proposed pathways; 2) CEF1 is highly inducible and can achieve quite high rates; 3) CEF1 is critical for maintaining ATP levels under stress; 4) the NDH complex likely pumps protons, like its homolog in the mitochondrion, Complex I. Finally, we demonstrate the energetic importance of CEF1 in stress responses in plants and CO_2 pumping in algae.



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Citrate-Binding Site in Proteorhodopsin Involves Two Lysines in the First Cytoplasmic Loop

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Citrate-induced aggregation of native proteorhodopsin (pR) from octylglucoside solutions, observed at citrate concentrations as low as ~15 mM (pH 5.5-7), is fully reversible by removal of the citrate and raising of the pH in the presence of detergent. The aggregation is specific for several tricarboxylic acid (TCA) cycle intermediates, including citrate, isocitrate, and cis-aconitate. The aggregation is not merely salting-out, since it is inhibited by phosphate. The citrate binding site on pR includes lysines 57 and 59, two of the four cationic residues in the first intracellular loop. That is, citrate no longer causes precipitation of pR when these lysines are mutated. The loss of citrate sensitivity is nearly complete for the quadruple mutant R51Q/R53Q/K57Q/K59Q, even with 1 M citrate. The double mutant K57Q/K59Q behaves nearly the same as the quadruple mutant. With the single-site R51Q mutant, on the other hand, there is only a ~2-fold increase in the citrate concentration required to induce pR aggregation. Thus, the 2 arginines in the 1st intracellular loop play only a minor role in the citrate binding site Lysines 57 and 59 are conserved among pR sequences from genetically diverse /gamma/-proteobacteria. This conservation likely reflects a physiological function for the citrate binding site of pR, i.e. a structure-function relationship. Aggregation of pR occurs at citrate concentrations only a bit above the endogenous concentration, ~10 mM, that has been measured inside thriving bacterial cells. This suggests that pR activity may be allosterically regulated by intracellular citrate. This would be consistent with recent observations in other labs, that bacteria expressing pR carry out light energy transduction only under starvation conditions that would be expected to decrease intracellular tricarboxylic acid concentrations.

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Predicting the Reaction Coordinates of Millisecond Light-Induced Conformational Changes in Photoactive Yellow Protein

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Understanding the dynamics of large-scale conformational changes in proteins still poses a challenge for molecular simulation, as such processes occur on long time scales. The transition path sampling method aims to sample reactive paths connecting two stable states. We used transition path sampling to investigate the mechanisms underlying the millisecond timescale partial unfolding transition in the photocycle of the bacterial sensor Photoactive Yellow Protein. This reaction is characterized by loss of alpha-helical structure and solvent exposure of the chromophore binding pocket. Advanced analysis methods predict the best model for the reaction coordinates of each step in the unfolding reactions as well as tentative transition states.

We find that the unfolding of the alpha-helical region 43-51 is followed by sequential solvent exposure of Glu46 and the chromophore. Solvent exposure of the chromophore can also occur first, but is a dead-end route. Which of these two residues is exposed first, is correlated with the presence of a salt bridge that is part of the N-terminal domain.